

TRACE MINERALS (Zn, Cu, Se, and Mn) AND THE ACQUIRED IMMUNE RESPONSE IN CATTLE

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INTRODUCTION

The immune system consists of two main branches; the innate and the adaptive immunity. These two components have specific functions, but work integrated to protect the animal from infections. Trace minerals (TM) are crucial for the development of an adequate immune response in cattle, especially in stressed animals, since some of these micro-elements activate or are structural components of metabolic pathways, DNA replication, antioxidant protective systems, etc. The benefits of administering trace minerals on animal health and performance have been previously assessed in dairy (Harrison et al., 1984; Machado et al., 2013), and beef cattle (Arthington et al., 2014; Berry et al., 2000; Genther, and Hansen, 2014; Richeson and Kegley, 2011). Several studies have evaluated the effects of injectable TM on the immune function of cattle (Arthington and Havenga, 2012; Chirase et al., 1994; Clark et al., 2006; Droke and Loerch, 1989), some of which will be discussed in this manuscript.

Field and experimental studies focused on improving the TM status of feeder calves have shown positive effects of administration of TM improving feed efficiency, decreasing morbidity and treatment costs and improving performance and production traits. Our studies at University of Georgia revealed that administration of injectable TM (Se, Zn, Cu and Mn) concurrently with modified-live virus vaccination in dairy and beef calves resulted in earlier and increased antibody titer and leukocyte proliferation upon stimulation with viruses compared to the control group. Therefore, addition of injectable TM supplements to calf management protocols might represent a promising tool to improve livestock health on commercial farms.

THE IMMUNE SYSTEM IN CATTLE

Innate Immunity

The innate immune system represents the first line of defense against a pathogen before the adaptive system can develop the appropriate response. The innate immunity is not antigen specific and does not include anamnestic or memory response. Components of nonspecific immunity are present both prior to and following antigen exposure and they do not discriminate against most foreign substances. These components include natural barriers (skin, epithelial lining, anti-microbial substances, enzymes, etc.), complement proteins, and white blood cells (granulocytes, macrophages) that engulf and eliminate pathogens (phagocytosis and killing).

Further, the innate immunity also includes type I interferon (IFN) antiviral response and natural killer cells (NKC) which are lymphocytes able to destroy infected cells. Granulocytes are also called polymorphonuclear leukocytes (PMN), mainly neutrophils. The main function of neutrophils is to monitor for infection to perform phagocytosis and killing of pathogens. Neutrophils identify pathogens by the recognition of pathogen-associated molecular patterns (PAMPs) using specific receptors, called Toll-like receptors. Microbes have molecules not typically found in mammalian cells (e.g. double-stranded RNA, CpG DNA sequences, and unusual carbohydrate residues), so that neutrophils are able to detect these molecules to eliminate the infectious agents. After binding of PAMPs to Toll-like receptors, neutrophils activate and initiate the phagocytosis and killing. Microbial destruction by phagocytosis involves the production of reactive oxygen species (ROS) which are bactericidal.

Adaptive Immunity

The adaptive or acquired immunity has the capacity to recognize specific antigens and has memory. The primary components of the adaptive immune system involve humoral (antibody production by B lymphocytes) and cell mediated immunity (developed mainly by CD4⁺ helper T cells, CD8⁺ cytotoxic T cells and WC1⁺ γδ T cells).

B-cells mature in bone marrow and are released into blood, where they circulate and populate lymphoid tissues. These cells also act as antigen presenting cells (APC) so that they can recognize antigens and present them to helper T cells, which enhance further antibody production. B lymphocytes activate and undergo proliferation and differentiation, a process termed “clonal expansion”. Each clone of B lymphocytes can recognize a specific target antigen. Production of antibodies by B lymphocytes following interaction with the antigen requires input of interleukins-2 (IL-2), IL-4 and IFN-γ. These cytokines also cause formation of B memory cells from the activated B-cell population. Antibodies are Y-shaped proteins, containing a constant (C) region and a variable (V) region. The V region contains the antigen binding site. The constant region is assembled from distinct genes that yield different antibody isotopes, such as IgG, IgM, IgD, IgA and IgE. Each of these isotopes has different physical properties and specific roles in the animal’s immune system. The roles of antibodies in adaptive immune response include: neutralization of microbe and toxins, opsonization and phagocytosis, antibody-dependent cellular cytotoxicity, and complement activation.

T-cells are produced in the bone marrow and mature in the Thymus (hence the name “T”-cell). T-cells are responsible for “cell-mediated immunity”. Similar to B cells, clonal expansion and differentiation of T-cells results in the development of effector and memory T-cells. During cell-mediated immunity, T lymphocytes recruit and stimulate phagocytic (nonspecific) activity as well as participate in direct lysis of infected cells (e.g. viral infected cells). Helper T-lymphocytes (CD4⁺) orchestrate the adaptive immune response through the production of cytokines and costimulatory molecules, while cytotoxic T-cells (CD8⁺) have the capacity to destroy infected cells (e.g. during virus infection).

ROLE OF TRACE MINERALS IN THE IMMUNE RESPONSE

Nutritional status, and particularly mineral levels, have been demonstrated to impact cattle health and performance (Enjalbert et al., 2006; Galyean et al., 1999; Underwood and Suttle, 1999). Trace minerals such as Zinc (Zn), Manganese (Mn), Copper (Cu), and Selenium (Se) are important for optimal immune function (Chirase et al., 1991; Percival, 1998; Underwood and Suttle, 1999) and growth (Spears and Kegley, 2002) in cattle, particularly in highly stressed, and newly received feeder calves (Duff and Galyean, 2007). Zinc contributes with the structure and function of more than 2,500 enzyme systems involved in metabolism (Andreini et al., 2009; Cousins and King, 2004). Zinc activates the enzyme superoxide dismutase, which plays a crucial role in stabilizing cell membranes against reactive oxygen species (ROS) (Bonaventura et al., 2015; Haase and Rink, 2014). Zinc is involved in DNA replication through the actions of ribonucleotide reductase, and is necessary for lymphocytes proliferation and differentiation. Zinc's major roles in the immune response involve signaling and adhesion of neutrophils and macrophages (Bonaventura et al., 2015), production of pro-inflammatory cytokines by monocytes (Rink and Kirchner, 2000), regulation of IL-2 secretion, signal transduction for T cell activation, clonal expansion, differentiation and T_H cells polarization (Haase and Rink, 2014), B-cell function, and antibody production (Pinna et al., 2002; Tomlinson et al., 2008).

Copper is important in the mitochondrial metabolic cascades for energy production to supply different organs, including those of the immune system (Failla, 2003). Copper also plays a role in superoxide dismutase activity and neutralization of ROS (Maggini et al., 2007), and contributes to the process of phagocyte killing (Linder, 1991). Ceruloplasmin is a copper-containing enzyme whose production increases dramatically during inflammation in response to the necessity of scavenging oxygen radicals released by immune cells (Percival, 1998). In rodents, copper deficiency is associated with decreased IL-2 production, lymphocyte proliferation and T cells counts (Bala and Failla, 1993; Bonham et al., 2002; Klotz et al., 2003; Linder and Hazegh-Azam, 1996; Minatel and Carfagnini, 2000; O'Dell, 1993; Pan and Loo, 2000; Percival, 1998). Similarly, studies in cattle fed a copper-deficient diet showed a significant reduction in B-lymphocytes and impaired neutrophil activity (Cerone et al., 1998).

Selenium appears to be very important to the migration of neutrophils into tissues and subsequent inflammation (Maddox et al., 1999). Selenium is a component of the enzyme glutathione peroxidase that inactivates ROS production and prevents released ROS from causing cellular damage (Maddox et al., 1999; Neve, 1991). Selenium deficiencies have been associated with depressed neutrophil migration and killing ability, and reduced B-cell response and antibody production. Moreover, Se supplementation enhanced both humoral and cell-mediated and immune responses (Maggini et al., 2007). The level of Se in tissues and blood affected the total IgM levels and BHV1-specific antibody titers after challenge (Reffett et al., 1988). Evidence that Mn plays a role in the immune system is limited. However, Mn has an essential function in removing ROS produced by active phagocytic cells (Tomlinson et al., 2008).

EFFECT OF INJECTABLE TRACE MINERALS (ITM) ON THE IMMUNE RESPONSE TO VACCINATION AGAINST BOVINE RESPIRATORY DISEASE (BRD).

Bovine respiratory disease (BRD) has a major impact on the profitability of the dairy and beef industries in North America, resulting in substantial economic losses that exceed \$1 billion annually (Griffin, 1997; McVey, 2009). The infectious agents most consistently implicated in BRD include *Bovine viral diarrhea virus* (BVDV), *Bovine herpes virus 1* (BHV1), *Bovine respiratory syncytial virus* (BRSV), *Parainfluenza 3 virus* (PI3V), *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. Appropriate biosecurity measures and vaccination program are crucial to prevent and control BRD.

Arthington and Havenga (2012) assessed the effect of administration of ITM on the humoral immune response after BRD specific MLV vaccination in cattle. That study demonstrated that ITM given concurrently with viral vaccination enhanced the production of neutralizing antibodies to BHV1 in beef calves. Additionally, recent studies have shown that treatment with ITM concomitantly with MLV vaccination induced a faster BVDV-specific antibody response in newly received, highly stressed calves (Roberts et al., 2015).

A growing body of evidence suggests that both humoral and cell mediated immune (CMI) responses are critical in protection against viral agents involved in BRD (Collen and Morrison, 2000; Howard 1990; Nobiron et al., 2003). A more complete evaluation of the immune responses induced by MLV vaccination requires the use of methods to assess both humoral (antibody response) and cellular effector mechanisms (recall antigen induced proliferation and induction of IFN- γ as the core Th1 cytokine).

A study was performed at University of Georgia to evaluate the effect of an injectable trace mineral (ITM) supplement containing zinc, manganese, selenium, and copper on the humoral and CMI responses to vaccine antigens in dairy calves receiving a modified-live viral (MLV) vaccine containing BVDV, BHV1, PI3V and BRSV (Palomares et al., 2016). A total of 30 dairy calves (3.5 months of age) were administered a priming dose of the MLV vaccine containing BHV1, BVDV1 & 2, BRSV, PI3V, and an attenuated-live *Mannheimia-Pasteurella* bacterin subcutaneously (SQ). Calves were randomly assigned to 1 of 2 groups: (1) administration of ITM SQ (ITM, Multimin 90® Multimin USA®, n = 15) or (2) injection of sterile saline SQ (Control; n = 15). Three weeks later, calves received a booster of the same vaccine combination SQ, and a second administration of ITM, or sterile saline, according to the treatment group. Throughout the study, the calves grazed Bermuda grass (*Cynodon dactylon*) and Fescue grass (*Festuca arundinacea*) with no access to mineral supplementation. Animals had access to hay (Bermuda grass and Fescue grass) and water *ad libitum*. Additionally, calves received 2.7 kg/head/day of concentrate supplement (Bulk Cattleman's Special; Godfrey's Warehouse; Madison-GA; Palomares et al., 2016) divided into two meals.

In this study, calves had adequate liver tissue concentration of all the study trace minerals assessed at all sampling dates according to standard reference values (Herdt and Hoff, 2011). Administration of ITM resulted in increased concentrations of liver Se (on days 21 and 56), Cu (on day 56) and Mn (on day 56) compared to saline injected calves (Palomares et al., 2016).

Administration of ITM concurrently with MLV vaccination resulted in higher antibody titers to BVDV1 on day 28 after priming vaccination compared to the control group ($P = 0.03$). This higher BVDV1 specific humoral immune response was associated with both higher production of antibody (Fig. 1A) and a numerical tendency of greater proportion of sero-conversion (≥ 4 -fold increase compared to day 0) to BVDV1 on day 28 in the ITM group (12/15; 80.0%) compared to the control group (8/15; 53.3%; $P = 0.13$; Fig. 1B).

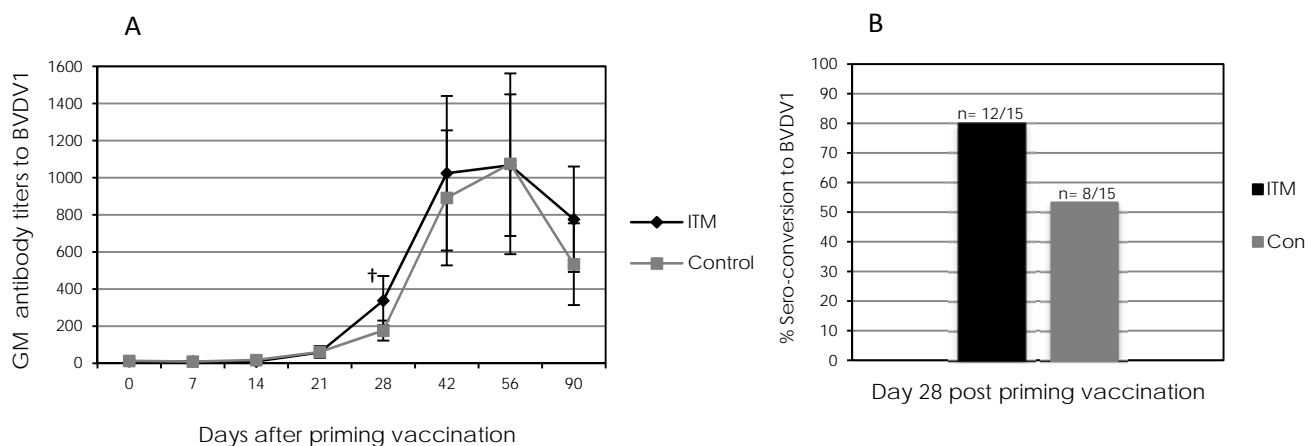


Figure 1. Geometric means serum neutralizing (SN) antibody titers to BVDV1 (A), and proportion of sero-conversion or ≥ 4 -fold increase in SN antibody titer to BVDV1 on day 28 relative to the day of priming vaccination (B) in dairy calves that received injectable trace minerals (ITM) or saline (Control) concurrently with a MLV vaccine. [†]Significant differences between groups for each day ($P = 0.03$).

This quicker BVDV-specific antibody response elicited by an MLV vaccine delivered concurrently with ITM might be of significant value by conferring earlier protection following vaccination. This could be particularly important in newly received, highly stressed cattle that are at high risk of respiratory viral infections. A rapid increase in serum neutralizing antibodies against BVDV after vaccination may be beneficial to prevent infection and disease development. The antibodies may neutralize extracellular virus particles, inhibiting attachment of the virus to host cells, and contribute to antibody dependent cell mediated cytotoxicity (Forthal, 2014). Similarly, previous reports have shown that ITM enhanced humoral immune response to pathogens of clinical significance in cattle production, including BVDV (Roberts et al., 2015), BHV-1 (Arthington and Havenga 2012), *E. coli* (Panousis et al., 2001) and *Pasteurella*

haemolytica (Droke and Loerch, 1989). In the study performed by Arthington and Havenga (2012), administration of trace minerals concurrently with an MLV vaccine to steers induced a significant increase in BHV-1 serum neutralizing antibody titers on days 14, 30, and 60 post-vaccination compared to the base line titers on day 0 and to the titers in the saline injected steers.

Calves treated with ITM showed an earlier enhancement in mononuclear leukocyte proliferation to BVDV1 following vaccination compared to the control group (peak of leukocyte proliferation occurred 14 days later). Proliferation of mononuclear leukocytes after BVDV stimulation tended to be higher on day 14 after priming vaccination in calves treated with ITM than in the control group ($P = 0.08$; Fig. 2A). Calves that received ITM showed higher mononuclear leukocyte proliferation to BRSV stimulation on day 7 after priming vaccination compared to the control group ($P = 0.01$; Fig. 2B). Moreover, calves in the ITM group also had an enhanced production IFN- γ by PBMC after stimulation with BRSV on day 21 after priming vaccination compared to day 0 ($P < 0.01$).

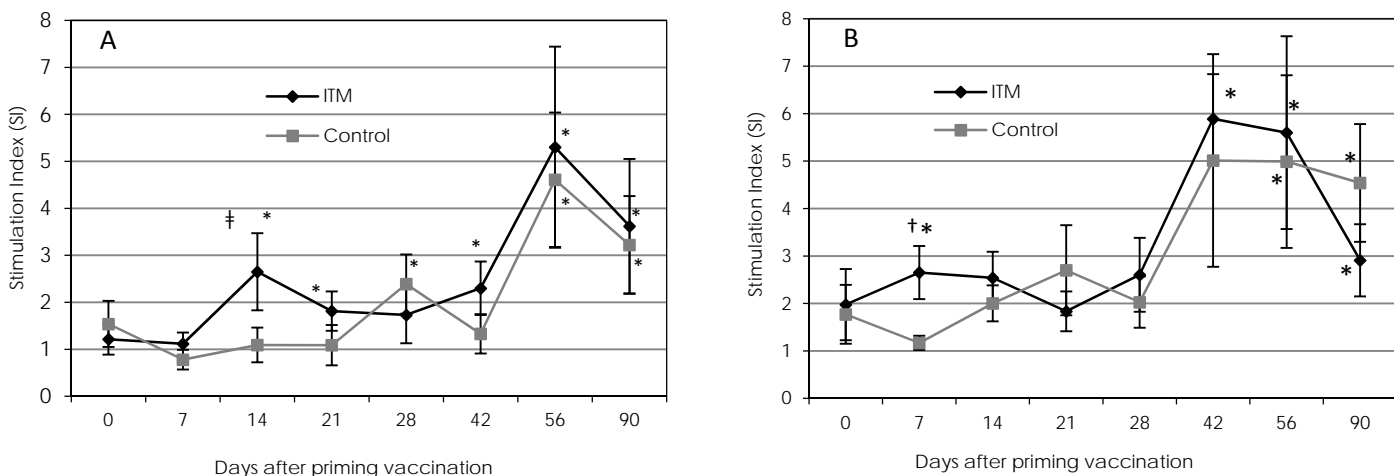


Figure 2. Peripheral blood mononuclear leukocyte proliferation (expressed as stimulation index; SI) in response to BVDV1 (A), and BRSV (B) in dairy calves injected with a trace mineral supplement (ITM) or saline (Control) concurrently with administration of a MLV vaccine. * Value differs significantly from the value on day 0 for each group (3A: $P < 0.05$; 3B: $P < 0.01$).[‡]Suggests a tendency of higher leukocyte proliferation compared to the control group (3A, $P = 0.08$).[†]Significant difference between groups (3B, $P = 0.01$).

Adequate supply of Zn, Cu, Se and Mn has been documented to be essential for cell signaling and cytokine production during lymphocyte activation (Puertollano et al., 2011; Spears, 2000). These trace minerals are fundamental elements in the structure and function of several metalloproteins that participate in general housekeeping processes involved in cellular clonal expansion including metabolic cascades for energy production, DNA replication and transcription, as well as protection against ROS (Failla,

2003). The enhancement of these general cellular functions might be contributing to the higher leukocyte proliferation response observed in the ITM group.

Trace minerals are crucial for the development of an adequate immune response in cattle, especially in stressed animals. Field and experimental studies focused on improving the trace mineral status of stressed feeder calves have shown positive effects of ITM improving feed efficiency, decreasing morbidity and treatment costs and improving performance and production traits (Arthington et al., 2014; Berry et al., 2000; Clark et al., 2006; Genther, and Hansen, 2014; Richeson et al., 2011). The results of the current study support our hypothesis, and recapitulate the findings of previous studies demonstrating the benefits of trace mineral supplementation on the immune response to MLV vaccines in cattle. This suggests that addition of ITM to calf management protocols might represent a promising tool to improve livestock health on commercial farms.

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